

# Crystal Structures of Vascular Endothelial Growth Factor in Complex with Fab Fragments

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## INTRODUCTION

Vascular endothelial growth factor (VEGF) is a cell-selective mitogen for vascular endothelial cells [1]. As a potent angiogenic factor it is required for the development of the vascular system and has been implicated in pathological processes such as tumor growth, metastasis, diabetic retinopathy and rheumatoid arthritis [2]. Neutralizing antibodies against VEGF are capable of suppressing tumor growth in a murine model [3] and will potentially be very useful therapeutic agents to regulate excessive vascularization.

VEGF is a homodimeric glycoprotein, which occurs naturally in four different isoforms consisting of 121, 165, 189 or 206 residues. The three longer isoforms are capable of binding to heparin and are found in complex with heparan sulfate containing proteoglycans in the extracellular matrix. Plasmin cleavage of VEGF<sub>165</sub>, the most abundant isoform, yields an N-terminal fragment consisting of 110 amino acids. This 'receptor-binding' fragment is capable of binding to the two receptors of VEGF, the fms-like tyrosine kinase (flt-1) and the kinase domain receptor (KDR), with wild type affinity [4].

Recently, the structures of a truncated receptor binding fragment covering residues 8 to 109 has been solved in its unbound state [5], in complex with the second domain of the flt-1 receptor [6], and in complex with the humanized version of antibody A4.6.1. [7]. Based on the structure of VEGF in complex with the Fab-fragment, two affinity improved antibodies (Y0313 and Y0317) were derived using phage display.

## RESULTS AND DISCUSSION

The receptor binding domain of VEGF in complex with Y0317 crystallizes in spacegroup P2<sub>1</sub> with cell parameters of  $a=89.1 \text{ \AA}$ ,  $b=66.4 \text{ \AA}$ ,  $c=138.8 \text{ \AA}$ , and  $\beta=94.7^\circ$ . A full dataset with a maximum resolution of  $2.5 \text{ \AA}$  and an  $R_{\text{sym}}$  of 7.6% was collected at ALS, beamline 5, in December 1997. The previously solved structure of VEGF in complex with an antibody fragment was used to solve the structure. The current R-value is 25.3% with a R-free of 30.1% and refinement is in progress.

The first X-ray quality crystals of VEGF<sub>165</sub>, the most abundant isoform of VEGF, were obtained by forming a complex with the Fab fragment Y0313. These crystals diffract to a maximum resolution of  $2.8 \text{ \AA}$  and belong to space group P2 with cell parameters of  $a=107.6 \text{ \AA}$ ,  $b=65.8 \text{ \AA}$ ,  $c=123.8 \text{ \AA}$ , and  $\beta=93.4^\circ$ . A complete dataset of good quality with a R-sym of 7.4% was collected in December 1997 at ALS, beamline 5. One complex consisting of one Fab fragment bound to each side of a VEGF dimer was expected in the asymmetric unit. The structure was solved by molecular replacement, using the constant domains and the variable domains of an Fab fragment as a search model. A model of the receptor binding domain of VEGF could be placed unambiguously in a resulting difference density map. Refinement of the structure is in progress, the current R-value is 27% with an R-free of 34% using all data between  $2.8 \text{ \AA}$  and  $20 \text{ \AA}$ .

These two structures, together with the structure of the parent Fab in complex with the receptor binding domain of VEGF [7], will help us to understand the different binding affinities towards VEGF of the three different Fab fragments.

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